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13. Abstract *(Maximum 200 Words)*

The primary function of the Gulf War Illness (GWIC) consortium is to identify the pathobiological mechanisms of Gulf War Illness. The ultimate goal is to discover and characterize biomarkers of Gulf War illness and then identify targeted treatment strategies. The GWIC allows for the development of multidisciplinary collaborations targeting suspected brain-immune signaling alterations in GWI. The GWIC consortium central hypothesis identifies chronic neuroinflammation as an end result of initial glial activation and subsequent priming of glial responses that cause a chronic activation loop of stronger and longer proinflammatory signaling effects between the immune system and the brain. The GWIC includes both clinical (human) and preclinical (animal and cell) studies and researchers in the 10 funded sub-studies. These studies are incorporating sufficient overlap of scientific content area to inform each other in a bench-to-bedside-to-bench approach. Results to date from the preclinical (animal) studies suggest a strong neuroinflammatory component to the illness model and provide leads for treatment development approaches in the animal model before translation to the clinic. Final institutional review approval for clinical studies has recently been obtained and recruitment for clinical studies will begin shortly.

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INTRODUCTION

Background. More than 20 years after the 1991 Gulf War, 25-32% of the nearly 700,000 U.S. troops who served in the war still suffer from the debilitating symptomatic illness known as Gulf War Illness (GWI) (RAC, 2008, 2014, IOM, 2010). A growing body of evidence indicates that GWI is associated with diverse central nervous system (CNS) and immune alterations, but the specific pathobiological processes driving GWI symptoms have not been clearly elucidated (Zhang et al., 1999; Sullivan et al, 2003; Heaton et al., 2007; Toomey et al., 2009; Whistler et al., 2009; Broderick et al., 2011; Chao et al., 2011; Sullivan et al., 2013). Animal studies indicate that a chronic CNS inflammatory state can develop in response to an insult—chemical injury, infection, or physical trauma—that mobilizes CNS defense systems via activation of glia, the brain's primary immune response cells, and release of chemical messengers that precipitate a complex of "sickness behavior symptoms" identified by measures of impaired memory and learning, increased pain sensitivity, and persistent fatigue, a symptom complex similar to that of GWI (Banks & Lein, 2012; Watkins et al., 2007; 2009; Zhang et al., 2010). Recent studies have also demonstrated CNS inflammatory effects of GW-related exposures and additional immune and cellular processes that plausibly explain the mechanisms contributing to the full spectrum of GWI symptoms (Milligan et al., 2009; Rivest et al., 2009; Spradling et al., 2011).

Consortium Management and Expertise. This multidisciplinary collaboration brings together established GWI researchers, and leading experts in brain-immune processes associated with neurotoxicology and neuroinflammation, damage to white matter and axonal transport, immunology, and immunogenetics. This team has designed a body of interrelated studies linked together by a cohesive model of brain-immune interactions as the basis for GWI. The consortium is led by Dr. Kimberly Sullivan, at Boston University (BU), whose extensive background in GWI research includes contributions in identifying effects of Gulf War exposures on brain structure and function (Sullivan et al., 2003; Sullivan et al., 2013). BU serves as the Coordinating Center for the Gulf War Illness Consortium (GWIC) and provides the Administrative and Data Management Cores (figure 1). The consortium also includes a Preclinical Core, consisting of experts at five sites who are working collaboratively to characterize the persistent neurological and immune effects of GW exposures at the physiological, tissue, and cellular levels. This is done in parallel with human studies conducted by the Clinical Core at three recruitment sites (and two additional laboratory sites) to characterize the specific profile of brain, immune, and genetic measures that distinguish veterans

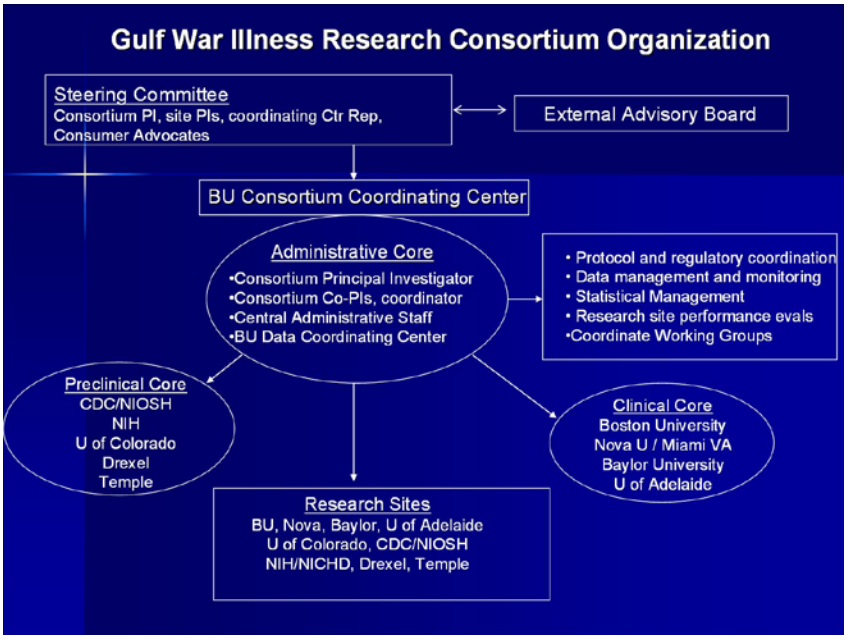


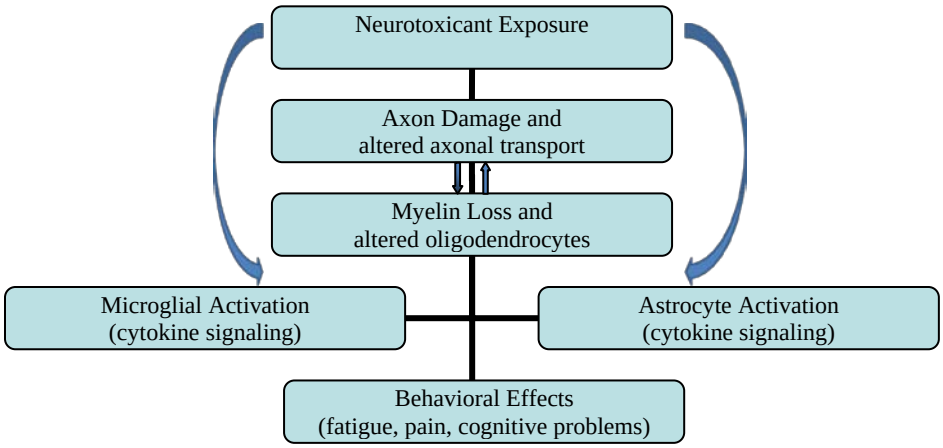
Figure 1. GWI Consortium schematic Organizational Chart

with GWI from healthy controls. The GWIC Steering Committee and External Advisory Committee monitors research progress and findings, and advises on research modifications and follow-up.

Objective. The primary objective of the Boston GWI consortium is to provide a cohesive understanding of the pathobiological mechanisms responsible for the symptoms of GWI in order to provide a rational and efficient basis for identifying beneficial treatments and diagnostic markers.

Research Plan. The consortium is undertaking a coordinated series of clinical and preclinical studies aimed at providing a comprehensive understanding of the pathobiology of GWI. This includes clinical case-control studies conducted in parallel at 3 subject recruitment sites—Boston, Miami, and Central Texas—that include a total of 300 Gulf War veterans. Clinical assessments include a) advanced neuroimaging protocols (MRI, DTI, fMRI, PET) that assess brain volumetrics, white matter integrity, and CNS inflammatory indicators, b) neuropsychological assessment of cognitive function, c) blood levels of cytokines and other immune signaling molecules, d) genetic expression of immune markers, e) pilot assessment of cerebrospinal fluid levels (CSF) of cytokines and neurotransmitters (in subgroup of Boston cohort), f) immunogenetic markers of innate immune responsivity, f) longitudinal assessment of brain-immune measures (Texas cohort only). Parallel preclinical studies will evaluate persistent effects of GW neurotoxicants *in vitro* and in rodent models of GWI. Preclinical studies will evaluate cellular effects of GW neurotoxicants on a) axonal transport, b) glial cytokine production, c) neurotransmitter signaling, d) myelination, and e) oligodendrocyte proliferation. Animal studies will determine the effects of GW exposures on: a) priming and maintaining glial activation, differentiating effects on astrocytes vs. microglia, b) glial activation in relation to development of learning impairment and chronic pain sensitivity, c) brain and blood levels of proinflammatory cytokines, and d) genetic expression of immune and inflammatory markers in brain and blood. Findings from clinical and preclinical studies will be compared and used to identify specific brain-immune pathways that can be targeted for intervention by a variety of glial modulating and other currently available treatments. Treatment compounds will be tested in animal models to determine their effectiveness for resolving or ameliorating the pathobiological processes associated with GWI. Figure 1 represents the hypothesized mechanisms for GWI that will be tested by this planned series of preclinical and clinical experiments.

Figure 1. Schematic Representation of Hypothesized GWI Mechanisms



The GWI consortium central hypothesis identifies chronic neuroinflammation as an end result of initial glial *activation* and subsequent *priming* of glial responses that cause a chronic activation loop of stronger and longer proinflammatory effects between the immune system and the brain. Figure 2 below represents the integrated theory of GWI that will be tested in the consortium studies.

More specifically, (1) the role of glia and resultant release of proinflammatory cytokines can lead to chronic pain and diminished cognitive processing, (2) axonal transport deficits and myelin alterations can lead to cognitive functioning difficulties, and (3) mitochondrial damage can lead to chronic fatigue-like illness. As support for this model, the current Boston investigators have found that GW veterans with known OP exposures showed significantly worse performance on information processing speed of sustained and selective attention, visual memory, and altered mood compared with less exposed GW veterans, and GW veterans with PB exposure showed significantly lower performance on a task assessing executive system functioning. In addition, this team of researchers found that symptomatic treatment-seeking GWVs showed worse performance on measures of attention, memory, visuospatial functioning, and mood alterations (Sullivan et al., 2003). Correspondingly, two recent MRI studies have shown lower white matter volumes in sarin-exposed GW veterans (Chao et al., 2011; Heaton et al., 2007). Neuronal and myelin breakdown products can take years to be cleared from the CNS due to Wallerian degeneration and die-back mechanisms may cause microglial activation and corresponding innate immune system activation as the microglia are considered the immune sentinels of the CNS (Milligan et al., 2009; Rivest, 2009; Vargas, 2007).

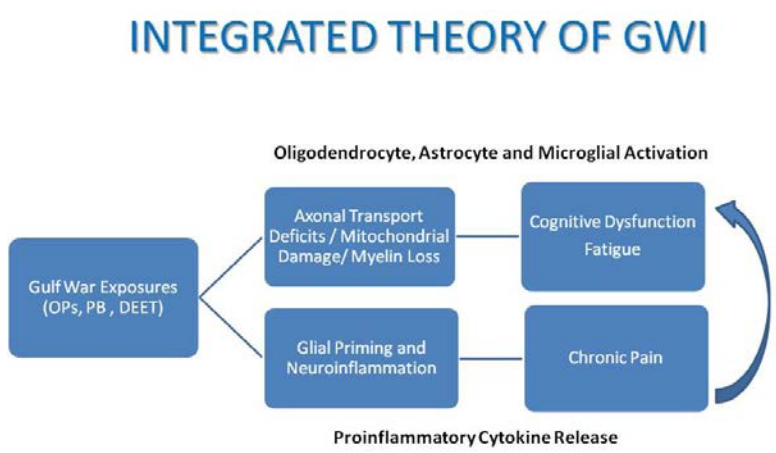


Figure 2. Integrated Theory of GWI

Behaviorally, the symptoms of fatigue, memory loss, and attentional deficits reported by GW veterans are similar to those seen in patients with diseases of the white matter that are also mediated by brain-immune interactions and cytokine signaling such as multiple sclerosis and chronic fatigue syndrome (CFS)(Brimacombe et al., 2002; Zhang, 1999; Baraniuk et al., 2005; Pantoni, 2009). Increased sensitivity to pain has also been related to proinflammatory cytokine signaling in the CNS in fibromyalgia and CFS and is often referred to as central sensitization (Arnett et al., 2012). Recent studies of GW veterans with chronic pain have also reported central sensitization of pain on functional MRI imaging (Gopinath et al., 2012). When considered in relation to reported increased rates of proinflammatory cytokines in blood samples from GW veterans with CFS when compared with either healthy GW veterans or civilians with CFS, the results suggest that neuron-glial and innate immune alterations appear to be associated with GWI and warrant further studied as biomarkers of illness (Brimacombe et al., 2002; Zhang et al., 1999).

The ultimate goal of the GWIC consortium is to identify the pathobiological mechanisms and validated biomarkers of GWI that can be easily translated into targeted future clinical treatment trials. The overall aims of this integrated multidisciplinary consortium scientific focus are to (1) To identify validated markers of GW illness by using state of the art neuroimaging, behavioral, genetic and blood markers of neuroinflammatory activation in both clinical and preclinical models that will elucidate targeted and validated treatment strategies (2) To create a Neuroinflammation Risk Profile for GWI (3) To identify viable mechanistic treatments based on identified pathophysiological pathways of GWI that have been validated in preclinical treatment models.

BODY

The approved statement of work for the entire study period is below:

STATEMENT OF WORK

Table 1. Brain-Immune Interactions as the Basis of Gulf War Illness: Gulf War Illness Consortium

Task 1. Obtain necessary authorization prior to initiation of human subjects' and animal studies research (months 1-8)
1a. Attend pre-award meeting with CDMRP GWIRP program staff
1b. Obtain final Institutional Review Board (IRB) approval for clinical research sites at Boston University School of Public Health (BUSPH), Baylor University and Miami VA/Nova University for protocols and advertisements
1c. Obtain final DOD Human subjects Research Protections Office (HRPO) approvals
1d. Obtain data use agreement from Hines VA for stored blood sample study
1e. Obtain final protocol approval by the respective Institutional Animal Care and Use Committees (IACUC) approval for the preclinical animal research sites at Center for Disease Control/NIOSH, National Institutes of Health, Drexel University, Temple University and University of Colorado
1f. Complete hiring of necessary staff and ensure all mandatory IRB and IACUC research related trainings are completed by all staff members
Task 2. Preparation for consortium clinical studies (months 1-9)
2a. BUSPH Data Coordinating Center (DCC) will create website, data collection forms, specimen tracking system and databases for the entire consortium including all preclinical and clinical sites.
2b. Develop manuals for the neuropsychological testing protocol, imaging protocols, specimen collection protocols and recruitment.
2c. Train researchers and staff on protocols and quality control measures for the clinical and preclinical studies.
2d. Obtain stored blood samples from Hines VA study and send to Miami VA for analysis.
Task 3. Preparation for consortium preclinical studies (months 9 - 24)
3a. Prepare rat dosing models at CDC and distribute to other sites at NIH, Drexel, Temple and U-Colorado for planned studies of axonal transport, myelin integrity and learning and pain assessments.
3b. Develop co-cultures of rodent oligodendrocytes in cell culture chambers for electrical stimulation of axons and development of myelination in vitro at NIH.
Task 4. Perform preclinical cell and animal studies (months 9-42)
4a. Assess for axonal transport integrity in rodent and cell models exposed to either GW-relevant neurotoxicants or cytokines (Drexel - 30 Sprague Dawley rats, Temple - 27 Sprague Dawley rats).
4b. Assess for myelin integrity in rodent and cell models exposed to either GW-relevant neurotoxicants or cytokines (NIH – 624 NIH/S mice and 208 rats).
4c. Assess whether persistent priming of neuroinflammation occurs chronically with GW-relevant neurotoxicants and intermittent corticosterone exposure to model the

chronic nature of GWI (CDC – 100 C57BL/6 mice).
4d. Assess the relative contributions of astrocytes and microglia in rodent GWI neuroinflammatory models in order to identify which glial markers will provide the best candidate "drugable" targets (CDC 40 C57BL/6 mice; 40 ALDH1L1 mice; 40 B6.129-Cx3CR1 mice).
4e. Assess the relationship between behavioral testing of learning and memory and enhanced pain, in rodent GWI neuroinflammatory models by assessing hippocampal functioning with a fear conditioning task (U-Colorado – 120 rats).
4f. Compare central and peripheral markers of neuroinflammation in brain tissue and blood samples from GWI neuroinflammatory rodent models (CDC – 60 rats, Nova).
4g. Compare the effectiveness of several relevant preclinical treatments for GWI in cell and animal studies, including inflammatory glial activation modulators, antioxidants, and neuroprotective peptides (Drexel, Temple, CDC, U-Colorado)(20 animals per treatment).
Task 5. Screening, recruitment and assessment of Gulf War veterans from three sites (months 9-42)
5a. Obtain informed consent from potentially eligible GW veterans
5b. Assess subjects by obtaining demographics, medical history, self-report questionnaires, neuropsychological testing, brain imaging and blood draw and saliva samples.
5c. Upload neuroimaging data to BUSPH for post-processing of MR images and for data analysis.
5d. Score neuropsychological tests and upload summary data to DCC for entry, cleaning and analyses.
5e. Send blood and saliva samples to Nova University for analysis of cytokine and chemokine panels and cortisol measurements.
5f. Send additional saliva samples to University of Adelaide for genetic polymorphism analysis
5g. Conduct preliminary analyses of clinical data
Task 6. Recruitment and assessment for Boston CSF and PET studies (months 24-42)
6a. Perform lumbar punctures to obtain cerebrospinal fluid markers of neuroinflammation in 50 GW veterans.
6b. Perform positron emission tomography (PET) scanning with novel EAAT2 ligand in partnership with RIO pharmaceuticals in 15 GW veterans.
6c. Perform FDG-PET scan imaging with 30 GW veterans after a computerized CPT cognitive challenge task.
Task. 7. Interim Analyses, Grant Submission, and Annual Reporting (Months 18-42)
7a. Data entry of all questionnaires, evaluations and quality control measures will be ongoing
7b. Interim Statistical analyses of data obtained from cognitive evaluations, blood markers, neuroimaging and questionnaire data will be performed periodically.
7c. Grant submissions to relevant funding agencies for further collaborative studies based on initial results and preliminary data targeted toward treatment strategies will be ongoing.
7d. Annual reports of progress will be written.
Task 8. Final analysis and Report Writing (months 42-48)
8a. Statistical analyses comparing brain MRI volumetrics, cognitive functioning, health symptom report and cytokine/chemokine markers in veterans with and without GWI

	8b. Statistical analyses of correlations between clinical and preclinical neuroinflammatory markers of GWI models
	8c. Perform longitudinal assessments of imaging, cognitive, health symptom and cytokine functioning in veterans with and without GWI
	8d. Perform validation analysis studies of identified biomarkers of GWI using an unrelated sample of stored blood and cognitive health symptom data from a prior CSP study.
	8e. Write final study report
	8f. Present findings at scientific meetings
	8g. Prepare manuscripts for submission
	8h. Write grant proposals based on consortium findings and identified treatment avenues for GWI.

The statement of work for year 1 is inclusive of Tasks 1-5 above. The statement of work for year 1 primarily describes the completion of the start-up phase of the 10 sub-studies including obtaining local and funder institutional review approvals for animal and clinical studies as well as establishing dosing models for cell and animal studies and finalizing clinical protocols for neuropsychological assessments, blood, saliva and CSF samples and neuroimaging sequences. In addition, in year 1, the plan was to recruit 15 study participants for the study protocol including cognitive evaluations, interviews, neuroimaging and specimen collection. Progress toward completing each task is listed below.

TASK 1. OBTAIN NECESSARY AUTHORIZATION PRIOR TO INITIATION OF HUMAN SUBJECTS' AND ANIMAL STUDIES RESEARCH (MONTHS 1-8)

Task 1a. Attend pre-award meeting with CDMRP GWIRP program staff
Due to delays in funding the consortium as a result of the government shutdown, the pre-award meeting was held in February 2013 and was considered a post-award meeting. The meeting included an overview of study hypotheses and plans as well as a review of the consortium administrative and core center structure. The Consortium PI, Dr. Sullivan and other steering committee members were present at the meeting in addition to CDMRP commanders, grants officer's representative (GOR) and administrative staff. Required External Advisory Board (EAB) meetings have also begun to meet with the first meeting being held in September 2014. The EAB provided helpful suggestions and comments for study progress and discussions for future meetings that will occur bi-annually during the consortium funding period.

Task 1b. Obtain final Institutional Review Board (IRB) approval for clinical research sites at Boston University School of Public Health (BUSPH), Baylor University and Miami VA/Nova University for protocols and advertisements
Institutional Review Board (IRB) approvals for clinical research have been obtained at Boston University Medical Campus, Miami VA Medical Center and Nova Southeastern University. Exempt status was obtained for the University of Adelaide site where de-identified saliva samples will be sent for genetic polymorphism analysis. Baylor University is not expected to start clinical studies until year 3 and will therefore obtain local IRB approval in the coming months.

Task 1c. Obtain final DOD Human subjects Research Protections Office (HRPO) approvals

Boston University, Nova Southeastern University and Miami VA Medical Center local IRB approvals were submitted to HRPO for approval. It was determined that the Boston University site would require a research monitor due to the greater than minimal risk status of the protocol due to the lumbar puncture procedure that will be performed in the CSF sub-study. Dr. Neil Kowall was listed as the study monitor for the consortium and the HRPO application was resubmitted. After resubmitting to HRPO with this information, we have just recently received HRPO approval to begin subject recruitment for the planned clinical studies.

Task 1d. Obtain data use agreement from Hines VA for stored blood sample study

It was determined by the Hines VA that Boston University would need to submit to local IRB for exempt status approval in order to submit a request for a VA Data Use Agreement. Exempt status has been obtained through Boston University IRB and the request has been submitted to VA central office for approval for the data use agreement to release the de-identified stored blood samples for this validation study. Once the data use agreement is approved, Hines VA will send the de-identified blood samples to Dr. Klimas in Florida for analysis.

Task 1e. Obtain final protocol approval by the respective Institutional Animal Care and Use Committees (IACUC) approval for the preclinical animal research sites at Center for Disease Control/NIOSH, National Institutes of Health, Drexel University, Temple University and University of Colorado

The preclinical studies have 4 study sites requiring IACUC approvals including Temple University, University of Colorado, Center for Disease Control (CDC) and National Institutes of Health (NIH). All required local IACUC approvals have been obtained and the Department of Defense ACURO final approvals have also been obtained for all pre-clinical sites.

Task 1f. Complete hiring of necessary staff and ensure all mandatory IRB and IACUC research related trainings are completed by all staff members

The consortium Administrative Core at Boston University has hired its research assistant/research nurse and the other research sites have hired the appropriate post-doctoral associates, pre-doctoral students and research assistants required for each of the 10 sub-studies. All staff members have completed the required IRB, HIPAA and IACUC trainings to begin preclinical and clinical study work.

TASK 2. PREPARATION FOR CONSORTIUM CLINICAL STUDIES

The consortium coordinating center and Administrative Core at Boston University has led many monthly web and in-person meetings to prepare for the clinical studies kick-off once all institutional approvals were obtained. A significant amount of time and effort was devoted to obtaining all required study and test administration materials and to developing centralized web-based data collection forms for the consortium studies. Table 2 lists these planning meetings. Smaller working group

meetings were also held during the past year to plan for particular consortium topic areas. The Working Groups are described in Table 3.

Table 2. GWIC Monthly Planning and EAB Meetings

Date	Type of Meeting	Discussion item
February 5th, 2014	Webinar	➢ Progress on Statement of work ➢ data management planning ➢ clinical protocol planning
February 26th, 2014	Post-Award Meeting	➢ Met with DOD and EAB representatives and overviewed consortium research plans and structure
March 3rd, 2014	In-person Boston	➢ Data management discussion and update
March 5th, 2014	Webinar	➢ Progress on Statement of work ➢ data management plans ➢ clinical protocols
April 2nd, 2014	Webinar	➢ Pre-clinical study progress ➢ clinical study protocols ➢ steering committee meeting schedule
April 9th, 2014	In-person Boston	➢ Neuropsychological protocols ➢ In-person training schedule
May 7th, 2014	Webinar	➢ External advisory board member changes ➢ Data coordinating center updates ➢ IRB and IACUC status updates
June 4th, 2014	Webinar	➢ Consortium website preview ➢ IACUC and IRB status updates ➢ Dates for EAB meeting ➢ In-person training schedule discussion
June 23rd 2014	In-person	➢ Neuropsychological protocol discussion ➢ In-person training preparation
July 1st, 2014	Webinar	➢ DCC presentation on REDCap forms, surveys and telephone screening tool for discussion and finalization ➢ Discussion of exclusionary criteria for Kansas case criteria ➢ Update on pre-clinical study progress
August 11th-13th	In-person Boston	➢ Presentations and updates from all research investigators ➢ Detailed training for all clinical study research staff
August 21st, 2014	In-person Boston	➢ Finalizing REDCap forms and telephone screener
September 3rd 2014	Webinar	➢ Clinical protocol discussion and finalization ➢ IACUC and ACURO updates ➢ Pre-clinical study updates
September 15th, 2014	In-person Ft. Detrick	➢ Presentations by GWIC PI and research investigators to DOD and External Advisory Board and discussion
October 1st 2014	Webinar	➢ Recap of EAB meeting and suggestions ➢ Clinical and pre-clinical study updates

Table 3. Consortium Working Groups

Working Group	Tasks	Members
Data Management Service Group	Assist with QC issues, data cleaning and data management and sharing, website management.	Christine Chaisson, DCC Consortium PI, co-PIs
Statistics Service Group	Perform analyses and provides statistical planning and advice for study investigators and research site PIs.	Timothy Heeren, Christine Chaisson, Consortium PI/co-PIs
Translational Working Group	Forum for Intellectual property and material (IP) issues, translation of results into papers, abstracts, new grant submissions and how clinical and preclinical results can inform each other.	Michael Pratt – BU Tech Transfer office Consortium PI, co-PIs Research site PIs, RIO
Behavioral Studies Working Group	Plan imaging protocols and provide quality control for multiple imaging sites. Plan behavioral testing protocols and coordinate preclinical and clinical studies for comparability.	Drs. Sullivan, Killiany, Krengel, Toomey, Steele, Klimas, Coller, Hutchinson, Maier, Watkins
Histopathology Working Group	Plan tissue studies of proinflammatory, glial, axonal transport and mitochondrial markers in similarly dosed animal and cell models.	Drs. Baas, Black, O'Callaghan, Fields, Maier, Watkins
Immune Genetics Working Group	Plan and implement studies assessing brain-immune interactions involving glia and proinflammatory cytokines/chemokines through genetic SNPs and mRNA and miRNA protein studies.	Drs. Coller, Hutchinson, Klimas, Steele, Sullivan, Watkins, Maier

Task 2a. BUSPH Data Coordinating Center (DCC) will create website, data collection forms, specimen tracking system and databases for the entire consortium including all preclinical and clinical sites.

The Data Coordinating Center (DCC) at BUSPH has developed a website as a splash page for subject recruitment purposes and a secure website server to house study information and documentation. DCC staff also assisted with finalization of the study participant telephone recruitment screening tool and constructed an electronic participant screening tool using REDCap software. DCC staff also finalized data collection forms for study personnel use and built an electronic data capture system using REDCap web-based software. A customized participant tracking and appointment log system was also constructed with separate secure login for each study site. DCC also worked with consortium research investigators to design a customized bar-coded bio-specimen tracking and inventory system, and conducted in-person trainings with all clinical research staff for web-based screening, tracking, and data collection tools. This highly experienced data management team has designed standardized data collection, management and tracking tools to ensure the highest data quality. The cleaned data will be converted into analytic datasets for the centralized statistical programming and data analysis.

Task 2b. Develop manuals for the neuropsychological testing protocol, imaging protocols, specimen collection protocols and recruitment.

Manuals for the neuropsychological testing protocol, neuroimaging protocol and blood, saliva and CSF specimen collection and recruitment protocols have been compiled and finalized. Table 4 describes the full study protocol for the three study sites in Boston, Miami and Central Texas.

Table 4. Summary of Clinical Assessments and Tests Conducted at 3 Clinical Study Sites

	Boston		Miami		Texas		TOTAL
Data Collected	Cases	Controls	Cases	Controls	Cases	Controls	
Questionnaires (demographics, general health and symptoms,pain, fatigue, sleep, medical conditions, deployment/exposure history)	125	50	25	25	50	25	n = 300 (200 cases/100 controls)
Clinical evaluation (medical history, height, weight, supine/standing BP and pulse, psychiatric diagnostic assessment)	125	50	25	25	50	25	n = 300 (200 cases/100 controls)
Clinical lab tests (CBC, metabolic profile, lipid panel, TSH, ANA, RF)	125	50	25	25	50	25	n = 300 (200 cases/100 controls)
Research assays (plasma cytokines/chemokines, nanostring inflammasome genetic panel, and salivary cortisol)	125	50	25	25	50	25	n = 300 (200 cases/100 controls)
Neuroimaging (MRI volumetrics and relaxometry, DTI, fMRI)	125	50	-	-	50	25	n = 250 (175 cases/75 controls)
Neuropsychological assessment (executive function, attention, memory, psychomotor function, motivation, mood)	125	50	25*	25*	50	25	n= 300 (175 cases/75 controls)
Longitudinal assessment (clinical evaluation, clinical lab tests, plasma cytokines, neuroimaging, neuropsychological assessment)	-	-	-	-	50	25	n = 75 (50 cases/25 controls)

CBC=complete blood count, TSH=thyroid stimulating hormone, ANA=antinuclear antibodies, RF=rheumatoid factor, MRI=magnetic resonance imaging, DTI=diffusion tensor imaging * select neuropsychological tests will be done in Miami

Task 2c. Train researchers and staff on protocols and quality control measures for the clinical and preclinical studies

A detailed in-person training session was held in August 2014 at the Boston University Coordinating Center for all clinical research personnel who will be working directly with study participants to ensure adequate quality control of test administration and interview procedures among the study sites. All neuropsychological testing materials and survey instruments have been ordered, purchased and were distributed among the three clinical study sites at this meeting. Quality control measures will continue to be instituted and monitored by experienced Administrative Core investigators as the clinical studies proceed to ensure good inter-rater reliability and to reduce tester drift among the study sites.

Task 2d. Obtain stored blood samples from Hines VA study and send to Miami VA for analysis.

Boston University has submitted the VA data use agreement and required documentation to VA central office for review and approval before the stored blood samples can be sent from the Hines VA to the Miami VA for analysis of the planned biomarker validation study. This review outcome is still currently pending.

TASK 3. PREPARATION FOR CONSORTIUM PRECLINICAL STUDIES (MONTHS 9 - 24)

As previously described, monthly web meeting and working group meetings were ongoing during the past year to prepare for the planned preclinical studies and to coordinate overlap of the studies and to ensure that the same neurotoxicant dosing and exposure model of GWI was being used among the four preclinical study sites. The CDC site was tasked with comparing the mouse and rat models of GWI to ensure comparability for planned studies and to distribute dosed animals and animal tissue to the preclinical sites. The planning stage was successful as discussions progressed and initial studies began at the preclinical study sites. Preparation for the specific preclinical study sites are detailed below.

Task 3a. Prepare rat dosing models at CDC and distribute to other sites at NIH, Drexel, Temple and U-Colorado for planned studies of axonal transport, myelin integrity and learning and pain assessments.

Dr. O'Callaghan at the CDC site prepared and validated rat dosing models based on his initial mouse GWI dosing models from prior DOD funded studies. Dr. O'Callaghan's prior data showed that the sarin surrogate compound, diisopropyl phosphorofluoridate; (DFP) alone or in combination with PB and DEET caused a marked proinflammatory response in multiple brain regions. Chronic exposure to the glucocorticoid, corticosterone (CORT), at levels associated with high physiological stress, markedly enhanced the CNS proinflammatory response to DFP. These "priming" effects now have been shown to persist for at least a month post the CORT/DFP/PB/DEET initial exposure. While these data constitute a promising animal model for GWI, Dr. O'Callaghan generated additional data using the Sprague-Dawley rat as the animal model. For the consortium studies, an initial set of rats were dosed and sent to the University of Colorado to initiate behavioral studies with the rat GWI dosing model after a memorandum of understanding for shipping the animals was approved between CDC and University of Colorado. Initial study results are listed in the sub-studies below. Brain tissue from Dr. O'Callaghan's GWI exposure model of dosed rats and mice were also recently sent to Drexel University to initiate the planned histopathology studies of potential neuronal, axonal and glial pathology.

Task 3b. Develop co-cultures of rodent oligodendrocytes in cell culture chambers for electrical stimulation of axons and development of myelination in vitro at NIH.

Dr. Dipankar Dutta was hired as a postdoctoral associate in the NIH laboratory of Dr. Douglas Fields. Dr. Dutta has been trained in making the cell cultures from embryonic mice and rats for studies of myelination and the co-cultures are currently being developed in the cell culture chambers for the ongoing and planned oligodendrocyte and myelin studies.

TASK 4. PERFORM PRECLINICAL CELL AND ANIMAL STUDIES (MONTHS 9-42)

The preclinical studies started later than initially anticipated for the CDC site and those sites waiting for distribution of animals or tissue from the CDC site because an interagency agreement for the release of funds from DOD to CDC took approximately 8 months out of this first year to be released. Considering this delay in funding, a significant amount of work has been initiated at the 5 preclinical study sites to date. Specific progress to date is listed below for each of the sub-studies.

Task 4a. Assess for axonal transport integrity in rodent and cell models exposed to either GW-relevant neurotoxicants or cytokines (Drexel - 30 Sprague Dawley rats, Temple - 27 Sprague Dawley rats).

Studies to assess axonal transport and microtubule integrity have begun at the Philadelphia study sites (Drexel and Temple Universities). This research team is exploring the effects of GW neurotoxins and

cytokines on axonal transport and neuronal function *in vitro* and *in vivo*. Items completed to date for the axonal transport and microtubule integrity group include:

1) Obtained previously back-ordered neurotoxicant (DFP) from the chemical supplier Sigma.
2) A fluorescent mitochondrial stain, Mitotracker, has been tested in hippocampal neurons for use in the planned axonal transport live-cell imaging exposure studies.
3) The microtubule transport assay has been vastly improved and benchmark experiments are being conducted to establish data that will serve as a point of reference for our GWI neurotoxin studies
4) Conducted experiments investigating the protein CAMSAP2 and its binding to the minus-ends of microtubules under control conditions. A protocol to immunostain for CAMSAP2 has been optimized and this technique will be used as an additional outcome measure of how microtubule integrity is affected by Gulf War neurotoxicants.
5) Drexel investigators conferred with Dr. O'Callaghan at CDC regarding obtaining brain tissue from dosed animals for histopathology studies. The protocols for the immunohistochemistry studies have been worked out for all the necessary antibodies (APP, IgG, GFAP, MAP2, NeuN, Iba1 and ED-1), along with quantification criteria and techniques. Standard operating procedures have also been devised for the FJB and Nissl-Myelin staining techniques.
6) Initial work has begun on performing DFP and DFP + corticosterone (CORT) toxicity screen in hippocampal neurons to establish working concentrations of compounds, investigating the effects of DFP and DFP + CORT on mitochondrial transport, examining the effects of DFP and DFP + CORT on microtubule transport and staining and analysis of mouse tissue from *in vivo* studies.

Task 4b. Assess for myelin integrity in rodent and cell models exposed to either GW-relevant neurotoxicants or cytokines (NIH – 624 NIH/S mice and 208 rats).

As part of the initial studies on myelin integrity, it has been determined that there are functional cholinergic receptors on oligodendrocytes and the site-PI Dr. Fields is characterizing the type of receptor and how they change during development, using immunocytochemistry and live cell calcium imaging. Researchers have discovered that release of synaptic vesicles from perinodal astrocytes remodels myelin structure and exposes the node of Ranvier to disruption. This is relevant to immune attack on myelin and would be one of the consequences of GFAP upregulation that accompanies TBI and neurotoxicity. This has led to the identification of a possible biomarker for myelin damage (neurofascin 155) that will be investigated in CSF samples of veterans with GWI from the consortium biorepository. Dr. Fields is developing high sensitivity methods to detect this protein fragment (ELISA methods) for further study.

Task 4c. Assess whether persistent priming of neuroinflammation occurs chronically with GW-relevant neurotoxicants and intermittent corticosterone exposure to model the chronic nature of GWI (CDC – 100 C57BL/6 mice).

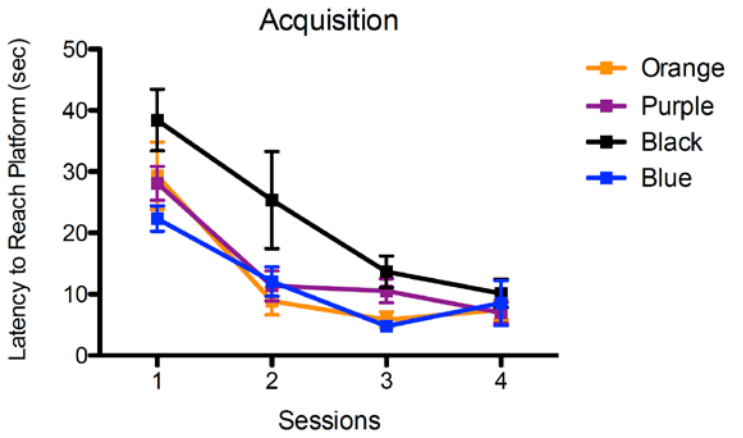
CDC investigators performed cytokine expression profiling in the Sprague-Dawley rat after DFP and CORT-DFP and determined that the enhanced cytokine expression seen in the GWI mouse model could also be generalized to the rat strain used in Dr. Maier's studies at the University of Colorado. Specific mouse model studies will begin shortly as well.

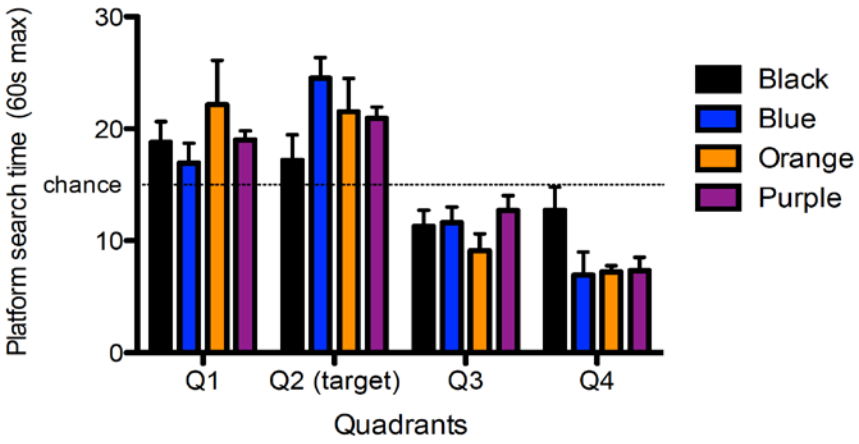
Task 4d. Assess the relative contributions of astrocytes and microglia in rodent GWI neuroinflammatory models in order to identify which glial markers will provide the best candidate "drugable" targets (CDC 40 C57BL/6 mice; 40 ALDH1L1 mice; 40 B6.129-Cx3CR1 mice).

Dr. Alicia Revitsky, was recruited and hired as a postdoctoral associate to work on the GWIC studies. Dr. O'Callaghan at CDC administered mice the known neurotoxicant, MPTP, to ALDH1-L1 BAC-TRAP mice to validate their use as a tool to assess astrocyte activation response with his Gulf War mouse dosing regimen. This experiment was successful as results obtained found mRNA expression profiling data in agreement with individual profiles from the CDC's own 20-yr historical data base. To our knowledge, no other toxicological use of this novel approach to assess cell-type specific gene expression currently exists in the toxicological literature. These results boost confidence that the BAC-TRAP mice can provide an mRNA signature unique to the GWI dosing model that will be employed.

Task 4e. Assess the relationship between behavioral testing of learning and memory and enhanced pain, in rodent GWI neuroinflammatory models by assessing hippocampal functioning with a fear conditioning task (U-Colorado – 120 rats).

The purpose of the proposed research was to determine whether the combination of exposure to corticosterone (CORT; mimicking "physiological stress") and DFP (mimicking sarin organophosphate exposure) produces cognitive deficits in rats and whether any such deficits are hippocampal in origin. To this end, Dr. O'Callaghan prepared 4 groups of rats for an initial study—Control, DFP, CORT, and DFP + CORT. For this initial study 6 mice were included in each group, and were shipped to University of Colorado after dosing at CDC. These rats were tested for the formation of spatial memory (hippocampal dependent) using the Morris water maze and for contextual fear memory after fear conditioning. Anxiety was also assessed as indicated by juvenile social interaction (JSE) ratings. The water maze data were striking as seen by the figures below (see figures 3-4; codes for the figures are: blue=controls, purple = CORT, orange = DFP, and black = DFP + CORT). Clearly, acquisition to escape by finding the safe platform in the spatial version of this task was severely impaired in the DFP + CORT group. However, escape was acquired. We then tested for memory of where the escape platform is located by testing the animals 24 hr later with the escape platform absent. If the subject remembers they are expected to spend their time in the quadrant where the platform had been located, the target quadrant was Q2, and all the groups except the DFP + CORT group spent much more time in this quadrant than would be expected by chance. That is, they remembered where the platform was located. The DFP + CORT group showed worse memory performance. The fear conditioning data were less clear and more subjects are needed in further studies





to make more definite conclusions. These data are highly encouraging and larger group sizes will make results and conclusions more evident. One difficulty encountered was that JSE testing suggested that all animal groups were anxious. This may have been related to the fact that the animals were shipped from Dr. O'Callaghan's laboratory after treatment, and so discussions are now ongoing to determine whether the "dosing" should be done directly in Colorado under Dr. O'Callaghan training and oversight rather than at the CDC laboratory for future planned studies.

Task 4f. Compare central and peripheral markers of neuroinflammation in brain tissue and blood samples from GWI neuroinflammatory rodent models (CDC – 60 rats, Nova).

This study plans to compare blood samples and brain tissue proteins from 60 dosed animals. This study will begin shortly when the CDC has a full complement of dosed study animals to send to NOVA University for analysis.

Task 4g. Compare the effectiveness of several relevant preclinical treatments for GWI in cell and animal studies, including inflammatory glial activation modulators, antioxidants, and neuroprotective peptides (Drexel, Temple, CDC, U-Colorado)(20 animals per treatment).

These important experiments will occur in the later years of the consortium when more information is known from the initial pathobiology studies in order to use those results to target appropriate choices for specific treatments to study.

TASK 5. SCREENING, RECRUITMENT AND ASSESSMENT OF GULF WAR VETERANS FROM THREE SITES (MONTHS 9-42)

Obtaining all necessary institutional approvals took slightly longer than initially expected and study recruitment has not yet begun. However, planning and finalization of study protocols for clinical studies have been completed. This included some minor changes to the Kansas Gulf War Illness criteria to allow for slightly less stringent exclusionary criteria for concomitant medical disorders within the past 5 years instead of ever having been diagnosed with the particular conditions. Additional questions were also added to the survey questions regarding past history of mild traumatic brain injury.

Task 5a. Obtain informed consent from potentially eligible GW veterans

DOD human research protection office (HRPO) approval has just been obtained to begin recruiting study participants so no participants have been recruited to date. Now that all institutional approvals have been obtained, recruitment will start immediately for the Boston and Miami sites. The Central Texas site will not begin recruitment until the next year to coincide with the longitudinal design of the Texas site of the consortium study. We do not anticipate this slight 3 month delay in recruitment start date to result in any problems with meeting recruitment goals in the coming year.

Task 5b. Assess subjects by obtaining demographics, medical history, self-report questionnaires, neuropsychological testing, brain imaging and blood draw and saliva samples.

No study participants have been recruited to date, however all study protocols for the clinical studies have been finalized and are listed in the tables below and in Table 1 above. **C**ognitive assessment data will be analyzed with neuroimaging data to assess for brain-behavior relationships in GWI.

Now that all study approvals have been obtained, we do not anticipate any difficulties with reaching recruitment goals for years 1 and 2 in the coming year.

Table 2. Neuropsychological Test Battery for GWI Consortium Study

Test Name	Description	Outcome Measure
I. Executive System Functioning		
Controlled Oral Word Association Test (COWAT)	Spontaneous generation of words from letters F, A and S and animals category.	Total correct words generated
Stroop Test (DKEFS)	Timed response requiring naming of ink color and inhibiting discordant color-names; measures fronto-executive, selective response and inhibition.	Total Errors
II. Tests of Attention, Vigilance and Tracking		
Trail-making Test (Reitan &Wolfson, 1985)	Timed connect-a-dot task to assess attention and motor control requiring sequencing (A) and alternating sequences (B).	Time to Completion
Continuous Performance Test (Connors')	Target letter embedded in series of distractors; to assess sustained attention and reaction time.	Reaction Time, Total Omission and Commission Errors
III. Tests of Motor Function		
Grooved Pegboard Test (Klove, 1963)	Speed of inserting pegs into slots using each hand separately; assesses motor coordination and speed.	Raw Score time to completion
Finger Tap Test (manual tapper)	Continuous tapping of computer key with alternate hands; assesses simple motor speed.	Number of taps
IV. Tests of Visuospatial Function		
Block Design Test (WAIS-IV)	Copy picture designs with blocks	Raw Score
Rey-Osterrieth Complex Figure Test	Copy of a complex figure	Total correct out of 36
V. Tests of Memory		
California Verbal Learning Test (CVLT- II; Delis et al., 2000)	List of 16 nouns from 4 categories presented over multiple learning trials with recall after interference; assesses memory and learning strategies.	Total Trials 1-5 Long Delay
Rey-Osterrieth Complex Figure Test	Immediate and delayed recall of a complex figure	Total recall out of 36
VI. Test of Motivation		
Test of Memory Malingering (TOMM)	Test of Memory Malingering (TOMM) is a 50-item visual recognition test designed to help distinguish malingering from genuine memory impairments	Total correct
VII. Mood measure		
Profile of Mood States	65 single-word descriptors of affective symptoms summed on six mood scales.	T – Scores

Table 3. Gulf War Illness Consortium - Survey and Questionnaire Instrument Descriptions

Name	Description
Demographics	Subjects report information on age, education, gender, ethnicity, marital status, GW duty service (active vs. reserve/National Guard), military rank and current military status.
SF36V	Veterans' version of the SF36 which assesses functional health-related quality of life in 8 domains and provides overall summary scores for physical and mental health status.
Kansas Gulf War and Health Questionnaire	Queries veterans about demographics, military and deployment history, and chronic symptoms and diagnoses required to ascertain Kansas GWI and CMI case status.
Medical Conditions	A checklist with 21 medical conditions that the subject is asked to rate if they have ever had the condition, how it was diagnosed (self or doctor) and when it was diagnosed.
Kansas Gulf War Experiences and Exposures	A questionnaire that assesses veteran-reported experiences and exposures during their deployment to the 1991 Gulf War.
Structured Neurotoxicant Assessment Checklist (SNAC)	The SNAC assesses the degree of past and current exposure to neurotoxicants during civilian and military occupations and includes questions pertaining to recent occupational and environmental exposures.
Pittsburg Sleep Quality Index (PSQI)	PSQI assesses sleep quality during the past month. It covers domains of sleep quality, latency, duration, efficiency, disturbances, medications and daytime dysfunction. Total global scores range from 0-21.
Multidimensional Fatigue Inventory Questionnaire (MFI-20)	20 item self-report fatigue instrument that covers general fatigue, physical fatigue, mental fatigue, reduced motivation and reduced activity.
McGill Pain Questionnaire	A pain questionnaire that includes 3 sections including what the pain feels like, change over time and strength of pain. Scores range from 0-78.

Task 5c. Upload neuroimaging data to BUSPH for post-processing of MR images and for data analysis.

Although study recruitment has not yet begun, when MRI images are obtained, they will be sent to Dr. Killiany at Boston University for post-processing and analysis. The MRI scans will be transferred electronically in either extended DICOM or par/rec format to the Center for Biomedical Imaging at Boston University School of Medicine. Each scan will undergo quality checking that consists of a visual inspection for the presence of noise or artifact as well as a review of scan parameters to ensure that the appropriate ones were used in the acquisition. Scans that fail the quality check will be rejected by the study and remediation discussed with the appropriate site investigator. Scans that pass the quality check will be entered the post-processing pipeline.

MRI Imaging: The scanning session will include: 1) Three plane TFSE scout scan, 2) a Sense reference Scan, 3) an accelerated high resolution MPRAGE scan acquired in the sagittal plane, 4) a multi-component T2 imaging sequence acquired in the axial plane, 5) a Diffusion Tensor Scan with 32 directions acquired in the axial plane, 6) a resting state functional magnetic resonance imaging

scan, and 7) a pCASL sequence obtained while the participant is at rest and 8) a High Angular Resolution Diffusion Imaging (HARDI DTI) scan.

Task 5d. Score neuropsychological tests and upload summary data to DCC for entry, cleaning and analyses.

No neuropsychological protocols have been collected to date for the planned consortium studies however, study staff at all sites have been trained for all test administration and standardized scoring procedures at the Boston Administrative Core. As study protocols are obtained and data is collected, quality control procedures will remain in place including double entry of data collection forms in the REDCap data collection website, built in range checks and quality control audits of all data collection by the Data Coordinating Center staff and the local BU Administrative Core neuropsychologists.

Task 5e. Send blood and saliva samples to Nova University for analysis of cytokine and chemokine panels and cortisol measurements.

No blood or saliva samples have been sent to NOVA Southeastern University yet for this study proposal. However, the analysis of cytokine, chemokine and cortisol measurements will include testing for neuroendocrine and immune alterations and for hypothalamic pituitary adrenal axis abnormalities. Specifically, blood samples will be sent to NOVA Southeastern University for analysis of proinflammatory cytokine and chemokines, monocyte markers (MCP-1), and nanostring analysis of mRNA and miRNA of proteins related to TLR4 functioning and glial activation including miR-155, miR-21 and miR-146. Multiplex Quansys ELISA system will be used with an existing cytokine platform created by Dr. Klimas' research laboratory. Dr. Klimas will measure 16 cytokines in plasma. Gene expression and pathways will also be assessed using an Agilent microarray system and quantitative real-time PCR for validation of differentially expressed genes.

Task 5f. Send additional saliva samples to University of Adelaide for genetic polymorphism analysis

No saliva samples have been sent to the University of Adelaide for genetic polymorphism studies to date. When the samples are sent, they will be analyzed for immune genetic markers of interest. Multiple genetic variants of important immune mediators and immune receptors involved in glial activation and how these genetic polymorphisms alter pathologies of GWI will be assessed. Variability in immune genetics targets of interest such as the gene encoding for IL-1B will be determined in the GWI populations and via comparison with a healthy control population related to both the development and severity of GWI symptoms. Genomic DNA will be isolated from the saliva samples and genotyped for targets of interest with custom-designed multiplex analysis.

Task 5g. Conduct preliminary analyses of clinical data

When preliminary data is available from the clinical study protocol collection, the BUSPH Data Coordinating Center will clean all data and prepare the datasets for statistical analysis in direct collaboration with the study biostatistician Dr. Tim Heeren and the study PIs.

KEY RESEARCH ACCOMPLISHMENTS

- Obtained final IRB and HRPO approvals for Nova Southeastern University, Miami VA and Boston University. Obtained Exempt status at University of Adelaide.

- Trained all clinical staff in-person on neuropsychological testing and structured clinical interviews and obtained all necessary equipment for three clinical sites.

- Data Coordinating Center has programmed online surveys in REDCap web data capture software, created a computer-assisted telephone screening and recruitment tool, developed a bar-coded specimen tracking system and a consortium website for subject recruitment.

- Obtained final protocol approvals by the respective IACUCs and ACURO for the preclinical animal research sites at CDC/NIOSH, NIH, Temple University and University of Colorado.

- Initial preclinical studies have begun and are showing promising results for:
  - Behavioral effects of the GWI neuroinflammatory model in rats
  - Axonal transport alterations in the in-vitro GWI model
  - Altered myelination and glial (astrocyte) signaling in the GWI model with a new potential biomarker identified that will be targeted for further study.

REPORTABLE OUTCOMES

Publications

Seichepine, D., Yee, M., **Janulewicz-Lloyd P., Sullivan, K & Krengel, M.** Chronicity of Health Symptoms in the Ft. Devens Cohort. International Neuropsychological Society, 42nd Annual Meeting Abstracts, Journal of the International Neuropsychological Society, Supplement 1, February 2014; 165.

Sullivan, K., Krengel, M., Janulewicz, P., and Chamberlain, J. An overview of toxicant exposures in Veteran cohorts from Vietnam to Iraq. In Amara, J. ,Hendricks, A.(eds) (2013) *Military medical care: From predeployment to post- separation,* Abingdon: Routledge.

Presentations

Sullivan, K., Klimas, N. Committee and Panel Discussion: 'how to discussion' for GWI Biomarker Research, Research Advisory Committee on Gulf War Veterans' Illnesses; Spring Meeting, Washington, DC, September, 2014.

Sullivan, K. Gulf War Illness Consortium Overview. Brain-Immune Interactions as the Basis of Gulf War Illness Consortium External Advisory Board (EAB) meeting with CDMRP commander and staff and EAB members, Ft. Detrick, MD, September, 2014.

O' Callaghan, J. Preclinical Cell/Animal Studies Overview. Brain-Immune Interactions as the Basis of Gulf War Illness Consortium External Advisory Board (EAB) meeting with CDMRP commander and staff and EAB members, Ft. Detrick, MD, September, 2014.

Baas, P. Axonal Transport Studies. Brain-Immune Interactions as the Basis of Gulf War Illness Consortium External Advisory Board (EAB) meeting with CDMRP commander and staff and EAB members, Ft. Detrick, MD, September, 2014.

Fields, D. Myelination Studies. Brain-Immune Interactions as the Basis of Gulf War Illness Consortium External Advisory Board (EAB) meeting with CDMRP commander and staff and EAB members, Ft. Detrick, MD, September, 2014.

Steele, L. GWI Case Definition. Brain-Immune Interactions as the Basis of Gulf War Illness Consortium External Advisory Board (EAB) meeting with CDMRP commander and staff and EAB members, Ft. Detrick, MD, September, 2014.

Klimas, N. Immune Genetics Studies and Consortium Biorepository. Brain-Immune Interactions as the Basis of Gulf War Illness Consortium External Advisory Board (EAB) meeting with CDMRP commander and staff and EAB members, Ft. Detrick, MD, September, 2014.

Killiany, R. Brain Imaging Studies Overview. Brain-Immune Interactions as the Basis of Gulf War Illness Consortium External Advisory Board (EAB) meeting with CDMRP commander and staff and EAB members, Ft. Detrick, MD, September, 2014.

O'Callaghan, J., **Sullivan, K.** Committee and Panel Discussion: 'how to discussion' for GWI animal research, Research Advisory Committee on Gulf War Veterans' Illnesses; Spring Meeting, Washington, DC, April, 2014.

Sullivan, K. Gulf War Illness Consortium Overview. Brain-Immune Interactions as the Basis of Gulf War Illness Consortium Post-award meeting with CDMRP commander and staff, Washington, DC, February, 2014.

Seichepine, D., Yee M., **Janulewicz Lloyd P**., **Sullivan, K.** & **Krengel, M**. Chronicity of Health Symptoms in the Ft. Devens Cohort. International Neuropsychological Society, 42nd Annual Meeting, Seattle, WA, February 2014.

Steele, L. Committee and Panel Discussion: 'how to discussion' for GWI Case Criteria Research Advisory Committee on Gulf War Veterans' Illnesses; Winter Meeting, Washington, DC, January, 2014.

Seichepine, D., Yee, M., **Janulewicz Lloyd P., Sullivan, K.**, Proctor, S., & **Krengel, M.** Traumatic Brain Injury and Health Status of Veterans from the 1990-1991 Gulf War. Boston University Second Annual Joining Forces TBI/PTSD Event, Boston, MA, December, 11, 2013.

Sullivan, K. RAC-GWVI Treatment Development Discussion. Research Advisory Committee on Gulf War Veterans' Illnesses; Summer Meeting, Washington, DC, June, 2013.

Grant funding

Drs. Sullivan and Krengel recently applied for a GWI grant as Co-Investigators with other Boston area researchers. This study is highly complementary with planned GWIC studies and was recently recommended for funding and is listed below.

Title: An in-vivo investigation of Brain Inflammation in Gulf War Illness with Integrated PET/MR imaging (PI: Loggia)
Supporting agency: Department of Defense (CDMRP/GWIRP GW130100)
Specific aims: The overarching objective of this study is to demonstrate the pathological occurrence of microglial activation in the brains of patients with Gulf War Illness and document the effects of this activation on Gulf War Illness symptomatology and brain physiology using new imaging approaches. The project's three specific aims are (1) to demonstrate *in vivo* activation of microglia in veterans with Gulf War Illness, (2) to demonstrate the association between microglial activation and alterations in brain physiology and anatomy, and (3) to demonstrate an association between microglial and neural activity with symptom severity; i.e., fatigue, pain, disability, depression, and anxiety.

In addition, Drs. Sullivan, Baas, Klimas, Fields, Coller, Toomey and Krengel also applied for several additional Gulf War Illness-related grants that will complement and build upon the current GWI consortium studies and, if funded, will utilize the valuable biorepository samples from the GWIC study for additional follow-on studies or further development of consortium study hypotheses. These submitted proposals are listed below.

- Drs. Baas, Sullivan and Coller submitted an invited grant application to CDMRP to use cutting-edge technology to provide immortal induced pluripotent stem cell (iPSCs) lines derived from GW veterans' blood samples that can be used to study many mechanistic hypotheses and to test many different treatment regimes for these ailing veterans. The specific aims are: (1) Develop human neurons or glial cells derived from human induced pluripotent stem cells (hiPSCs), originating from 15 GW veterans with GWI and 15 healthy GW veteran controls. (2) Develop a microtubule-based strategy to treat impaired nervous system functions in GWI.

- Drs. Sullivan and Klimas submitted an invited grant application with other GWI researchers to study the transforming growth factor-Beta (TGF-β) superfamily (SF) in stored blood samples from the GWIC biorepository due to its recent implication as a major player in the

neurodegenerative immune and energy metabolism disorders. The specific aims are: 1) to perform real-time polymerase chain reaction analysis (q-PCR) of the human TGF-β SF signaling pathway, mitochondria and inflammatory cytokines 2) Perform confirmation studies by measuring proteins of interest as determined in Aim 1 by using multiplex technology 3) Perform gene expression analysis and pathway data mining using an interactive software program s that results can be used for the development of targeted treatments.

- Dr. Sullivan and Toomey submitted an invited pilot treatment trial grant application to CDMRP to assess d-cycloserine as a potential treatment for the cognitive symptoms associated with Gulf War Illness. Specific Aims are 1) To compare efficacy of the novel therapeutic approach of DCS in improving cognitive functioning in GW veterans with GWI 2) To examine different time points in order to determine optimal timing of doses of DCS for positive effects on cognitive functioning 3) To compare efficacy of DCS in improving mood, health symptoms and quality of life measures in GW veterans with GWI.

- Drs. Sullivan, Fields, Krengel and Klimas submitted a letter of intent to submit a grant application in collaboration with other GWI researchers to study autoantibodies of central nervous system (CNS) biomarkers in peripheral blood sera of Gulf War veterans. Thus, we will attempt to identify peripheral markers of CNS damage in GWI. Study aims are to use this serum autoantibody to identify, quantify and standardize the method to determine presence of circulating IgG-class autoantibodies in sera of individuals with GWI compared to controls' cytoskeletal proteins associated with (1) neurogenesis, i.e., neurofilament triplet proteins (NFP), tubulin, microtubule associated protein-tau (tau protein) , and microtubule associated protein-2 (MAP-2); (2) myelinogenesis, i.e., myelin basic protein (MBP); and (3) astrogliogenesis, i.e., glial fibrillary acidic protein (GFAP), neurofascin 155 and S100B.

CONCLUSIONS TO DATE

This multi-institutional collaboration of highly qualified GWI researchers from public universities, federal agencies, and the private sector, provide an unprecedented opportunity to more fully elucidate the underlying pathobiology of Gulf War illness in one integrated model that once proven, will lead to focused treatment trials that can be quickly implemented.

The central hypothesis for the pathobiological mechanisms of GWI in this consortium includes chronic neuroinflammation as a result of initial glial *activation* and then *priming* of glial responses that cause stronger and longer responses that do not shut off the chemical cascade of proinflammatory cytokines and chemokines that cross-talk between the immune system and the brain. This could result in a lasting multisystem illness affecting many body systems, as seen in GWI.

Improved understanding of the role of glial activation in chronic pain states has given rise to rapidly expanding efforts to identify pharmaceuticals that specifically focus on glial functions. The growing availability of treatments of this type gives particular urgency to our efforts to determine the extent

to which glial activation and central cytokine activation explain the symptoms of GWI. In order to specifically address the research gaps outlined by the IOM and the RAC reports with regard to biomarker identification and pathobiology of GWI, this research team is characterizing disease symptoms and validating and improving pathobiological markers based on collective prior clinical and preclinical studies and leveraging longitudinal cohorts and stored blood samples with the ultimate goal of identifying targeted and effective treatments for GWI. Initial preliminary results suggest that the consortium animal model of GWI is correlated with behavioral alterations seen in clinical studies including altered visuospatial memory functioning. Other results have suggested a potential biomarker of myelin damage in GWI that will also be further targeted. As these preclinical models are further developed, they will be correlated with clinical studies to identify markers of the illness and targets for therapeutic intervention.

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